AF & IFW

Docket No.: G2000-700210

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant:	Judith K. Gwathmey et al.
Serial No:	09/990,705
Confirmation. No.:	3899
Filed:	November 21, 2001
For:	ISOLATION PROCEDURE AND OPTIMIZED MEDIA SOLUTION TO ENHANCE LONG-TERM SURVIVAL OF CELLS
Examiner:	Vera Afremova, Ph.D.
Art Unit:	1651

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Mail Stop Appeal Brief - Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 26th day of October, 2004.

Christopher R. Rhodes

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith are the following documents:

[X] Appeal brief Under 37 C.F.R. § 1.192 (3 copies)
[X] Return Receipt Postcard
[X] Check for $170.00

If the enclosed papers are considered incomplete, the Mail Room and/or the Application Branch is respectfully requested to contact the undersigned at (617) 395-7000.

A check is enclosed. If the fee is not sufficient, the Commissioner is hereby authorized to charge Deposit Account No. 50/2762. A duplicate of this sheet is enclosed.

Respectfully submitted,
Judith K. Gwathmey et al., Appellant

By: _____
Christopher R. Rhodes, Reg. No. 47,022
LOWRIE, LANDO & ANASTASI, LLP
Riverfront Office Park
One Main Street
Cambridge, MA 02142
(617) 395-7000

Date: October 26, 2004

752058

Attorney Docket No. G2000-700210

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant:	Judith K. Gwathmey et al.
Serial No.	09/990,705
Filed:	November 21, 2001
Title:	ISOLATION PROCEDURE AND OPTIMIZED MEDIA SOLUTION TO ENHANCE LONG-TERM SURVIVAL OF CELLS
Examiner:	Vera Afremova, Ph.D.
Confirmation:	3899
Art Unit:	1651

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that three (3) copies of this document are being placed in the United States mail with first-class postage attached, addressed to MAIL STOP APPEAL BRIEF - PATENTS, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on October 26, 2004.

Christopher R. Rhodes

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

11/01/2004 JADD01 00000011 09990705
01 FC:2402 170.00 OP

APPEAL BRIEF UNDER 37 C.F.R. § 1.192

Dear Sir:

This Appeal Brief is filed in response to the Advisory Action mailed on July 13, 2004 and in furtherance of the Notice of Appeal filed on August 26, 2004. This Appeal Brief is being filed in triplicate. A fee of $170 under 37 C.F.R. § 1.17(c) is enclosed herewith.

The **REAL PARTY IN INTEREST** can be found on page 2 of this Appeal Brief.

A statement of **RELATED APPEALS AND INTERFERENCES** can be found on page 2 of this Appeal Brief.

The **STATUS OF THE CLAIMS** can be found on page 2 of this Appeal Brief.

The **STATUS OF AMENDMENTS** can be found on page 2 of this Appeal Brief.

A **SUMMARY OF THE INVENTION** can be found on pages 2-3 of this Appeal Brief.

A concise statement of the **ISSUES** can be found on page 3 of this Appeal Brief.

A **GROUPING OF CLAIMS** can be found on pages 3-4 of this Appeal Brief.

The **ARGUMENT** can be found on pages 4-12 of this Appeal Brief.

An **APPENDIX OF CLAIMS** can be found on pages 12-19 of this Appeal Brief.

I. REAL PARTY IN INTEREST

The real party in interest is Gwathmey, Inc., a Massachusetts corporation, and assignee of the instant application.

II. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences known to Appellant, Appellant's legal representative, or the assignee of the instant application that will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

III. STATUS OF THE CLAIMS

Claims 1-26 were pending in the application as filed on November 21, 2002. Claims 14-26 were withdrawn in a response to a Restriction Requirement filed on March 12, 2003. Each of claims 1, 2, 12 and 13 was amended in an Amendment filed on November 17, 2003. Claims 1-13 are currently pending and are being appealed herein.

IV. STATUS OF AMENDMENTS

No amendment to the claims was filed or requested subsequent to the final Office Action mailed on April 26, 2004. The Appendix of Claims beginning on page 12 of this Appeal Brief incorporates all prior amendments.

V. SUMMARY OF THE INVENTION

Aspects and examples of the present invention are directed to methods for isolating and culturing cells, such as adult cardiomyocytes. See page 4, lines 9-11. For example, a method of isolating adult cardiac cells is disclosed. The method includes (a) obtaining a tissue sample from a subject, (b) successively exposing the tissue to a first solution with amounts of $CaCl_2$ decreasing from about 1-2 μM, comprising NaCl, HEPES, $MgCl_2$, KCl, and sugar at a pH of

approximately 7.4, (c) disassociating the tissue with an enzyme solution, (d) repeatedly resuspending the disassociated tissue into a second solution with amounts of $CaCl_2$ increasing from about 1-2 μM, comprising modified Earle's, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, and an antibiotic, at a pH of approximately 7.4 to obtain isolated cells. See page 4, line 23 to page 5, line 31.

VI. ISSUES

ISSUE 1

Whether the improper rejection of claims 1-13 under § 112, Second Paragraph, should be reversed when the claims, as presented, are definite.

ISSUE 2

Whether each of claims 1-13 is erroneously rejected under § 103 over Kruppenbacher (Naturwissenschaften 80, 132-134 (1993)) in combination with the ATCC catalog (page 522) and Kang (P.N.A.S. 91, 9886-9890 (1994)) where no *prima facie* case of obviousness has been established.

VII. GROUPING OF CLAIMS

For purposes of this appeal and only with respect to the improper rejections under § 103 and § 112, second paragraph, the claims do not stand or fall together, and, instead, each of claims 1-13 is believed to constitute a separately patentable group of claims.

A. **Group I:** Claim 1
B. **Group II:** Claim 2
C. **Group III:** Claim 3
D. **Group IV:** Claim 4
E. **Group V:** Claim 5
F. **Group VI:** Claim 6
G. **Group VII:** Claim 7
H. **Group VIII:** Claim 8
I. **Group IX:** Claim 9

J. **Group X:** Claim 10

K. **Group XI:** Claim 11

L. **Group XII:** Claim 12

M. **Group XIII:** Claim 13

VIII. ARGUMENT

For the reasons provided below, the Examiner's rejections are improper and should be reversed. Each of claims 1-13, as presented, is allowable.

A. The Rejection under § 112 is Improper Because Each of Claims 1-13 Meets the Definiteness Requirement of 35 U.S.C. § 112, Second Paragraph

Each of claims 1-13 (Groups I-XIII) is improperly rejected under § 112, second paragraph with respect to the recited amounts of calcium chloride and with respect to the amount of calcium in the modified Earle's. Each of claims 12 and 13 is improperly rejected under § 112, second paragraph as not indicating what volume is intended for ascorbic acid. These rejections should be reversed for the following reasons.

1. The Recited Amounts of Calcium Chloride are Definite

Claims 1 and 13 are each directed to isolation of adult cardiac cells in tissue. Each of claims 1 and 13 recites, in part, "successively exposing the tissue to a first solution with amounts of calcium chloride decreasing from about 1-2 μM." This step clearly refers to exposing tissue to a first solution with a selected amount of calcium chloride and then exposing the tissue to an additional solution having an amount of calcium chloride that is about 1-2 μM less than the selected amount of calcium chloride in the first solution. After successively exposing the tissue to a first solution with amounts of calcium chloride decreasing from about 1-2 μM, and after disassociation of the tissue with an enzyme solution, the disassociated tissue is repeatedly resuspended into a second solution with amounts of calcium chloride that increase from about 1-2 μM.

In view of the above, the steps of claims 1 and 13 that recite the use of solutions with "decreasing" and/or "increasing" amounts of calcium chloride are clear and definite, and each of

claims 1 (Group I) and 13 (Group XIII) meet the requirements of 35 U.S.C § 112, second paragraph.

Each of claims 2-12 (Groups II-XII) depends either directly or indirectly from claim 1 and meets the requirement of 35 U.S.C § 112, second paragraph for at least the same reasons as claim 1.

Accordingly, for the reasons presented above, each of claims 1-13 is improperly rejected under 35 U.S.C. § 112, second paragraph. Appellant respectfully requests reversal of the rejection.

2. The Recited Amount of Calcium in the Modified Earle's is Definite

With respect to the amount of calcium in the modified Earle's, as expressly recited in each of claims 1 and 13, it is within the ability of the person of ordinary skill in the art, given the benefit of the instant specification, to select suitable amounts of calcium chloride as starting and final points. It should be noted that claims 1 and 13 are not limited to any particular starting or final amounts of calcium chloride, but instead recite the use of decreasing or increasing amounts of calcium chloride based on an initial selected amount of calcium chloride.

Affirmation of the Examiner's rejection would unduly limit Appellant's claims to a specific amount of calcium. No such limitation should be required. A particular amount of starting or ending calcium chloride is not critical as long as the concentration of calcium chloride increases, or decreases, by about 1-2 μM. In particular, any suitable calcium concentration may be used along with modified Earle's so long as the tissue may be successively exposed to a first solution with amounts of calcium chloride that decrease from about 1-2 μM, and subsequent to dissassociation of the tissue with an enzyme solution, may be repeatedly resuspended in a second solution with amounts of calcium chloride that increase from about 1-2 μM calcium chloride.

Accordingly, the rejection is improper and should be reversed.

3. The Specific Amount Ascorbic Acid is Definite

With respect to the lack of volume for ascorbic acid, as recited in claims 12 and 13, the Examiner has not identified any requirement under 35 U.S.C § 112, second paragraph that an

amount of a reagent must be defined in terms of concentration, e.g., mass or moles per unit volume.

Because the specified amount of ascorbic acid is presented in standard terms of weight and measure, it is within the ability of the person of ordinary skill in the art, given the benefit of the instant application, to make any adjustments to reach a desired volume or concentration.

Accordingly, the rejection should be reversed.

B. Claims 1-13 are Patentable over Kruppenbacher in combination with the ATCC Catalog and Kang

Claims 1-13 are erroneously rejected under § 103(a) over Kruppenbacher (Naturwissenschaften 80, 132-134 (1993)) in combination with the ATCC catalog (page 522) and Kang (P.N.A.S. 91, 9886-9890 (1994)). This rejection is erroneous and should be reversed for the reasons detailed below.

1. No *prima facie* case of obviousness has been Established

It is well settled that three criteria must be met in order to establish a *prima facie* case of obviousness. First, the citation(s) must teach or suggest all of the claimed features. Second, there must be some specific suggestion or motivation, either in the cited citation(s) or in the knowledge generally available to one of ordinary skill in the art, to modify the citation(s). Third, there must be a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). As discussed below, none of these three requirements has been met in the present case, and, accordingly, no *prima facie* case of obviousness has been established.

a. No Suggestion or Motivation to Modify the Citations Exists

The Examiner has failed to identify any suggestion or motivation to modify Kruppenbacher with the teachings of the ATCC catalog or with Kang. It is well established that the examiner has the burden of presenting a convincing line of reasoning as to why the person of ordinary skill in the art would have found the claimed invention obvious in light of the teachings of the citations. See *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). When

the suggestion or motivation to modify the citations is not immediately apparent, it is the duty of the examiner to explain why the combination of the teachings is proper. See *Ex parte Skinner*, 2 USPQ2d 1788 (Bd. Pat. App. & Inter. 1986). The Examiner has not provided any convincing line of reasoning in support of the rejections, and has not explained why the combination of Kruppenbacher, Kang and the ATCC catalog is proper. This is not surprising considering Appellant has likewise identified no suggestion or motivation inherent in the citations to modify Kruppenbacher with the teachings of Kang or the ATCC catalog exists.

Kruppenbacher is directed to a method of isolating adult mouse cardiac myocytes using increasing amounts of calcium chloride. Kruppenbacher places adult mouse hearts in a perfusion buffer that includes 25 μM calcium chloride. See left column of page 133. These adult mouse hearts are then cut into pieces and chopped in the same perfusion buffer. The resulting tissue suspension is incubated in a water bath. After incubation in the water bath, the adult mouse cardiomyocytes are recalcified in three steps using 0.2, 0.5 and 1 mM calcium chloride.

The ATCC catalog was cited for the sole purpose of demonstrating the ingredients in the M199 medium recited in Kruppenbacher. The ATCC catalog makes no reference to the Kruppenbacher citation. Kang is directed to the use of isolated neonatal rat cardiac myocytes to measure contractility in the presence of long-chain polyunsaturated fatty acids. Kang also makes no reference to the Kruppenbacher citation.

There is no suggestion to modify Kruppenbacher with the teachings of Kang. In particular, there is no suggestion to modify the procedure of Kruppenbacher, designed for isolation of adult mouse cardiac myocytes, with a procedure designed to isolate neonatal rat cardiac myocytes, as taught by Kang. Accordingly, the Examiner's effort to modify Kruppenbacher is improper.

Kang also leads away from the subject matter of claims 1-13. Kang is directed to studies regarding contraction of neonatal rat cardiac myocytes, whereas claims 1-13 are directed to isolation of adult cardiac cells. Also, the HEPES solution of Kang, relied on by the Examiner (see page 5, third full paragraph, of the final Office Action), is used for performing contractility measurements of isolated neonatal rat cardiac myocytes and is not used to isolate the neonatal rat cardiac myocytes. Thus, Kang leads away from isolation of adult cardiac cells; Kang cannot be properly combined with Kruppenbacher; and Kang cannot be properly applied to claims 1-13.

In addition, Kruppenbacher discloses a solution having magnesium sulfate (see page 133, left column, first full paragraph), whereas Kang discloses a buffer solution having magnesium chloride (see paragraph bridging pages 9886 and 9887). The Examiner has not provided any objective evidence that there exists some suggestion or motivation in Kruppenbacher to substitute the sulfate salt of Kruppenbacher with the chloride salt in Kang.

In view of the above, no proper suggestion or motivation to combine Kruppenbacher with Kang has been provided, and, accordingly, no *prima facie* case of obviousness has been established. Appellant requests reversal of the rejection.

b. No Reasonable Expectation of Success has been Provided

It is well established that citations can only be modified or combined to reject claims as *prima facie* obvious if there is a reasonable expectation of success. *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). At least some degree of predictability is required. See M.P.E.P. § 2143.02.

The Examiner has not provided any objective evidence that Kruppenbacher's method of isolating adult mice cardiac myocytes could be modified with Kang's method of isolating neonatal rat cardiac myocytes to provide a successful result. Because of the distinct morphology between adult mice cardiac myocytes and neonatal rat cardiac myocytes, it is difficult to predict whether Kang's methods could be used in Kruppenbacher's methods to provide successful isolation of adult mice cardiac myocytes. Illustrative morphological and physiological differences between adult and neonatal cells are discussed in the Background section of the instant application. See, for example, page 3, lines 20-23.

In view of the above, because the Examiner has not provided any objective evidence that Kruppenbacher and Kang could be combined with a reasonable expectation of success, no *prima facie* case of obviousness has been established. Appellant requests reversal of the rejection.

c. The Combination of the Citations Fails to Teach or Suggest all the Claimed Features

Each of claims 1-13 is patentable over the combination of Kruppenbacher, ATCC catalog and Kang, because the combination of these citations fails to teach or suggest the subject matter

defined by any of claims 1-13. To establish *prima facie* obviousness of a claimed invention, all the claim elements must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). In addition, "all words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970).

When all the elements of each of claims 1-13 are properly considered, the combination of Kruppenbacher, ATCC catalog and Kang fails to teach the subject matter defined by each of claims 1-13.

As discussed above, Kruppenbacher is directed to a method of isolating cardiac myocytes that uses only increasing amounts of calcium chloride. Kruppenbacher places hearts in a perfusion buffer that includes 25 μM calcium chloride (see left column of page 133). These hearts are then cut into pieces and chopped in the same perfusion buffer. The resulting tissue suspension is incubated in a water bath. Nowhere does the method of Kruppenbacher expose the tissue suspension to water, as asserted by the Examiner in the Advisory Action. Incubation in a water bath is not the same as exposing the tissue to water. After incubation in the water bath, the cardiomyocytes are recalcified in three steps using 0.2, 0.5 and 1 mM calcium chloride. The method of Kruppenbacher thus exposes cardiomyocytes to a solution with 25 μM calcium chloride and then exposes the cardiomyocytes to solutions having 0.2, 0.5 and 1 mM calcium chloride. That is, the method of Kruppenbacher only exposes cardiomyocytes to increasing amounts of calcium chloride.

In contrast, the method defined by each of claims 1-13 successively exposes tissue to decreasing amounts of calcium chloride, disassociates the tissue with an enzyme solution, and then repeatedly suspends disassociated tissue in increasing amounts of calcium chloride. Kruppenbacher's failure to teach or suggest any exposure of tissue to successively decreasing amounts of calcium chloride leaves Kruppenbacher incapable of rendering any of claims 1-13 obvious.

In the Advisory Action, the Examiner asserts that Kruppenbacher somehow teaches the use of decreasing amounts of calcium chloride because Kruppenbacher mentions the term "recalcified." Appellant disagrees that such disclosure renders any of claims 1-13 obvious.

Even if Kruppenbacher does somehow reduce the levels of calcium, Kruppenbacher's mere recitation of the term "recalcified" is insufficient to provide the necessary teaching or suggestion to render any of claims 1-13 obvious. In particular, Kruppenbacher's recitation of the term "recalcified" does not provide any teaching or suggestion to successively expose tissue to a first solution with amounts of calcium chloride that decrease from about 1-2 μM, disassociate the tissue with an enzyme solution, and then repeatedly resuspend the disassociated tissue into a second solution with amounts of calcium chloride increasing from about 1-2 μM. Accordingly, Kruppenbacher remains deficient and incapable of rendering any of claims 1-13 obvious.

The above-noted deficiencies of Kruppenbacher are not cured by the ATCC catalog or Kang. In particular, there is no teaching or suggestion in the ATCC catalog regarding the subject matter defined by claims 1-13. Instead, the ATCC catalog was cited for the sole purpose of the ingredients in M199 medium. Both Kruppenbacher and the ATCC catalog disclose the M199 medium, which includes a magnesium sulfate salt that is clearly different than the magnesium chloride salt recited in each of claims 1-13.

Kang also fails to teach or suggest the use of decreasing amounts of calcium chloride to isolate adult cardiac cells. Kang uses a single perfusion buffer with a single concentration of calcium to measure contractility of neonatal rat cardiac myocytes (see paragraph bridging pages 9886-9887). Nowhere does Kang teach or suggest isolation of adult cardiac cells by successively exposing tissue to solutions of decreasing amounts of calcium chloride, disassociating the tissue with an enzyme solution and then repeatedly resuspending disassociated tissue in solutions including increasing amounts of calcium chloride. Thus, neither the ATCC catalog nor Kang cure the deficiencies of Kruppenbacher. Accordingly, claims 1-13 are patentable over the combination of Kruppenbacher, Kang and the ATCC catalog.

With particular reference to claim 2, the combination of Kruppenbacher, Kang and the ATCC catalog fails to teach or suggest the method of claim 1 further comprising the step of re-suspending the isolated cells approximately every 24 hours in a solution comprising modified Earle's, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, an antibiotic, and $CaCl_2$ at a pH of approximately 7.4. In particular, resuspending isolated adult cardiac cells every 24 hours is not disclosed, taught or suggested

by the combination of Kruppenbacher, Kang and the ATCC catalog. Accordingly, claim 2 is patentable over the combination of Kruppenbacher, Kang and the ATCC catalog.

With particular reference to claim 5, the combination of Kruppenbacher, Kang and the ATCC catalog fails to teach or suggest the method of claim 1 wherein the first solution is exposed to the tissue at approximately 37°C and at approximately 4 mL/min for 3 minutes. The recited flow rate of 4 mL/min for 3 minutes is not disclosed, taught or suggested by the combination of Kruppenbacher, Kang and the ATCC catalog. Kruppenbacher teaches a flow rate of 4 L/min per 4 hearts for 20 minutes (see page 133, left column, first full paragraph), and Kang teaches a flow rate of 20 mL/hour, or 0.33 mL/min (see page 9887, left column, line 3). Accordingly, claim 5 is patentable over the combination of Kruppenbacher, Kang and the ATCC catalog.

With particular reference to claim 7, the combination of Kruppenbacher, Kang and the ATCC catalog fails to teach or suggest the method of claim 1 wherein the first solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM D-glucose. In particular, no single solution used to isolate adult cardiac cells in any of Kruppenbacher, the ATCC catalog or Kang recites the particular concentrations of species listed in claim 7. Accordingly, claim 7 is patentable over the combination of Kruppenbacher, Kang and the ATCC catalog.

With particular reference to claim 11, the combination of Kruppenbacher, Kang and the ATCC catalog fails to teach or suggest the method of claim 1 wherein the enzyme solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM D-glucose. In particular, no single enzyme solution used to isolate adult cardiac cells in any of Kruppenbacher, the ATCC catalog or Kang recites the particular concentrations of species listed in claim 11. Accordingly, claim 11 is patentable over the combination of Kruppenbacher, Kang and the ATCC catalog.

With particular reference to claim 12, the combination of Kruppenbacher, Kang and the ATCC catalog fails to teach or suggest the method of claim 1 wherein the second solution comprises modified Earle's, L-glutamine, sodium bicarbonate at approximately 1250 mg/L, sodium pentothenate, creatine at approximately 328 mg/500mL, taurine at approximately 312

mg/500mL, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383 g/500mL, and an antibiotic at approximately 5% v/v at a pH of approximately 7.4 In particular, no solution used to isolate adult cardiac cells in any of Kruppenbacher, the ATCC catalog or Kang recites the particular concentrations of species, nor all of the species, listed in claim 11. Accordingly, claim 12 is patentable over the combination of Kruppenbacher, Kang and the ATCC catalog.

With particular reference to claim 13, the combination of Kruppenbacher, Kang and the ATCC catalog fails to teach or suggest use of the solutions defined in claim 13. In particular, the combinations and concentrations of the species listed in claim 13 are not taught or suggested by any Kruppenbacher, Kang and the ATCC catalog. Accordingly, claim 13 is patentable over the combination of Kruppenbacher, Kang and the ATCC catalog.

Because no *prima facie* case of obviousness has been established, Appellant requests reversal of the rejection.

IX. APPENDIX OF CLAIMS

1. (currently amended) A method of isolating adult cardiac cells comprising,

(a) obtaining a tissue sample from a subject,

(b) successively exposing the tissue to a first solution with ~~decreasing~~ amounts of $CaCl_2$ decreasing from about 1-2 μM, comprising NaCl, HEPES, $MgCl_2$, KCl, and sugar at a pH of approximately 7.4,

(c) disassociating the tissue with an enzyme solution,

(d) repeatedly resuspending the disassociated tissue into a second solution with ~~increasing~~ amounts of $CaCl_2$ increasing from about 1-2 μM, comprising modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, ~~fetal bovine serum,~~and an antibiotic, ~~and a fatty acid,~~ at a pH of approximately 7.4 to obtain isolated cells.

2. (currently amended) The method of claim 1, further comprising the step of re-suspending the isolated cells approximately every 24 hours in a solution comprising modified

Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, ~~fetal bovine serum,~~ an antibiotic, ~~a fatty acid acid,~~ and $CaCl_2$ at a pH of approximately 7.4.

3. (original) The method of claim 1, further comprising the step of incubating the isolated cells in a mixture of carbon dioxide and air.

4. (original) The method of claim 3, wherein the isolated cells are incubated at approximately 37°C.

5. (original) The method of claim 1 wherein, the first solution is exposed to the tissue at approximately 37°C and at approximately 4 ml/min for 3 minutes.

6. (original) The method of claim 1 wherein the concentration of $CaCl_2$ in the first solution decreases.

7. (original) The method of claim 1 wherein the first solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

8. (original) The method of claim 1 wherein the enzyme solution comprises a digestive enzyme.

9. (original) The method of claim 8, wherein the digestive enzyme is a protease or a collagenase.

10. (original) The method of claim 1 wherein the concentration of $CaCl_2$ in the second solution increases.

11. (original) The method of claim 1 wherein the enzyme solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

12. (currently amended) The method of claim 1 wherein the second solution comprises modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, Ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, ~~fetal bovine serum at approximately 10% v/v,~~ and an antibiotic at approximately 5% v/v, ~~a fatty acid at approximately 1 μM~~ at a pH of approximately 7.4.

13. (currently amended) A method of isolating adult cardiac cells comprising,

(a) obtaining a tissue sample from a subject,

(b) successively exposing at approximately 37°C the tissue to a first solution with ~~decreasing~~ amounts of $CaCl_2$ decreasing from about 1-2μM, comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM sugar at a pH of approximately 7.4,

(c) disassociating the tissue with an enzyme solution for approximately 8 minutes comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM sugar, to form disassociated cells,

(d) repeatedly resuspending the disassociated cells into a second solution with ~~increasing~~ amounts of $CaCl_2$ increasing to about 1-2μM, comprising modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, ~~fetal bovine serum at approximately 10% v/v,~~ and an antibiotic at approximately 5% v/v, ~~and a fatty acid at approximately 1 μM~~ at a pH of approximately 7.4 to form a solution of isolated cells,

(e) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C, and

(f) re-suspending the isolated cells approximately every 24 hours in a solution comprising modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, ~~fetal bovine serum,~~ an antibiotic, ~~a fatty acid,~~ and $CaCl_2$ at a pH of approximately 7.4 to obtain isolated cells.

14. (withdrawn) A method of cultivating isolated cells comprising, resuspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and $CaCl_2$ at a pH of approximately 7.4.

15. (withdrawn) The method of claim 14 wherein the solution comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1 μM, and approximately 1mM $CaCl_2$.

16. (withdrawn) A cell culture media for cells comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and $CaCl_2$ at a pH of approximately 7.4.

17. (withdrawn) The cell culture media of claim 16 wherein the media comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1 μM, and approximately 1mM $CaCl_2$.

18. (withdrawn) A method of isolating cells comprising,

(a) obtaining a tissue sample comprising cells from a subject ;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and nitrilotriacetic acid;

(d) incubating the tissue in a second solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme;

(e) incubating the tissue in a third solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme; and

(f) centrifuging the tissue to obtain isolated cells .

19. (withdrawn) The method of claim 18, further comprising the step of resuspending the isolated cells in a culture media comprising medium M199, BSA, ascorbic acid, taurine, carnitine, creatinine, insulin, and an antibiotic .

20. (withdrawn) The method of claim 19, wherein the culture media further comprises a fatty acid or magnesium.

21. (withdrawn) The method of claim 18, wherein the first solution comprises approximately 1-2 μM $CaCl_2$, approximately 120mM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96.

22. (withdrawn) The method of claim 18, wherein the second solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

23. (withdrawn) The method of claim 18, wherein the third solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl,

approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

24. (withdrawn) A method of isolating cells comprising,

(a) obtaining a tissue sample comprising cells from a subject ;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising approximately 1-2 μM $CaCl_2$, approximately 120mM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

(d) shaking the tissue at approximately 37°C for approximately 12 minutes;

(e) bubbling approximately 100% O_2 through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme; and

(h) centrifuging the tissue to obtain isolated cells.

25. (withdrawn) A method of isolating and cultivating human myocardial cells comprising,

(a) obtaining a tissue sample comprising myocardial cells from a human subject;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising approximately 1-2 μM calcium, approximately 120mM NaCl, approximately 5.4 mM KCl, approximately 5 mM

$MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

(d) shaking the tissue at approximately 37°C for approximately 12 minutes;

(e) bubbling approximately 100% O_2 through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2 μM, approximately 30 μM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2 μM, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 400 U/ml of a digestive enzyme;

(h) centrifuging the tissue to obtain isolated cells;

(i) repeatedly resuspending the disassociated cells into a second solution which comprises increasing amounts of $CaCl_2$, Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250 mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500 ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383 g/500 ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1 μM at a pH of approximately 7.4 to form a solution of isolated cells; and

(j) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C.

26. (withdrawn) A method of isolating and cultivating rodent myocardial cells comprising,

(a) removing the heart of a rodent;

(b) perfusing the heart with low calcium Tyrode's solution for approximately 3 minutes;

(c) perfusing the heart with an enzymatic solution for approximately 8 minutes;

(d) perfusing the heart with a low calcium solution for approximately 3 minutes;

(e) removing the ventricles;

(f) mincing the ventricles to isolate myocardial cells;

(g) mixing the cells in a low calcium solution;

(h) resuspending the cells in a solution comprising increasing concentrations of calcium; and

(i) resuspending the cells in culture media solution.

X. CONCLUSION

For the reasons provided above, each of the rejections is improper and should be reversed. Appellant respectfully requests reversal of the rejections and issuance of a Notice of Allowance.

Respectfully submitted,
For Appellant

By: _____

Ann Lampört Hammitte (Reg. No. 34,858)
Christopher R. Rhodes (Reg. No. 47,022)
Attorneys for Appellant
LOWRIE, LANDO & ANASTASI, LLP
One Main Street - 11th Floor
Cambridge, Massachusetts 02142 USA
Telephone: 617-395-7000
Facsimile: 617-395-7070

Date: October 26, 2004